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## **Development of cellular and humoral response against WT1 protein vaccination in mice**

To the Editor: Many anti-cancer vaccination strategies have been tested in mice and humans in the attempt to eradicate leukemia cells [1]. The vast majority of clinical trials are based on peptide vaccination which allows the induction of cellular response to specific tumor associated antigens [2]. WT1(Wilms tumor-1) gene is located on chromosome 11p13 and encodes a zinc finger transcription factor that plays an important role in cell growth and differentiation. WT1 was originally described as a tumor suppressor gene although many evidences demonstrated that it plays an oncogenic function in the setting of leukemia. WT1 protein represents an optimal tumor antigen since it is highly expressed in acute leukemias, myelodysplastic syndromes (MDS) and myeloproliferative neoplasms [3]. By contrast, it is expressed at very low levels in normal hematopoietic progenitors. Expression of the WT1 protein is restricted to a limited set of tissues, including the gonads, uterus, kidney, and spleen.

The success of a particular peptide vaccine to elicit an immune response is influenced by many parameters, including the presence of helper T cell epitopes, processing and presentation by professional antigen presenting cells (APCs), biodistribution, peptide length, peptide affinity, and route of administration. Recently Brayer [4] and colleagues published in this journal the results of WT1 peptide vaccination in AML and MDS. The conclusion from this study and many others based on WT1 peptide vaccination is that this strategy is safe, feasible but, at least in this study, it is not able to induce a consistent and measurable WT1 specific T cell response. In the majority of the clinical trials WT1 peptide elicited CD31 CD81 T cells. Additional trials showed that the combination of short and long peptides induced also CD31 CD41 T cells. Interestingly, it was shown that long peptide elicited the strongest immunological response against WT1. The clinical results are overall encouraging, describing several patients obtaining molecular remission, partial responses or stable disease. The main limits are the immune tolerance and immune evasion.

Two main strategies have been tested to overcome these limits, the use of longsequence peptides preferentially processed by APCs in the lymph node, circumventing some of the tolerance mechanisms, and the addition of adjuvant to stimulate APC. Here, we report the results of WT1 protein vaccination in mice. The complete WT1 murine coding sequence cloned in an expression vector (pGEX- 4T-1) together with GST protein has been amplified. The fusion protein GST-WT1 has been transfected in E.Coli and purified. Thirthy C57BL/6J mice have been utilized according to the scheme represented in Fig. 1 panel A. The first group (10 mice) was vaccinated performing a first injection with 30 lg of GST-WT1 protein150 lg of complete Freund adjuvant (AD) at Week 0. After 2 and 4 weeks, a second and third dose 30 lg of GST-WT1 protein150 lg of AD were injected. The second group (5 mice) was vaccinated with 30 lg of GST-WT1 protein only at week 0, 2 and 4. The third group (5 mice) was vaccinated with 30 lg of GST only plus AD at week 0, 2, and 4. The fourth group (10 mice) was treated with PBS only and used as control. After 2 additional weeks (weeks 6) 200.000 TRAMP-C cells, a singenic prostatic cancer cell line overexpressing WT1 were injected subcutaneously in all animals. After 8 weeks from the first injection half the mice were sacrificed to evaluate the immune response, both cytotoxic and humoral and the tumor burden, while half of them were sacrificed after 15 weeks to evaluate immune response, tumor borden, and organ toxicity. Dot blot analysis on mice serum showed the presence of IgG antibodies against WT1 after vaccination with GST-WT1 protein1AD and GST-WT1 protein alone. By contrast, the antibodies were not present after injection of GST 1AD and PBS. (Fig. 1 panel C and D). Furthermore, cytotoxicity of T cells was evaluated by 51Cr release test. In mice injected with GST-WT1 protein1AD the level of cytotoxicity was 30%62 compared to 2%60.5 (background level) in control mice. Finally, we examined the toxicity in organs which physiologically express WT1 al low levels: lymphonode, spleen, ovary, and kidney in vaccinated mice and controls. No toxicity was observed (Fig. 1 panel F). Hemocromocytometric analysis as well as BM smears (data not shown) excluded any kind of hematological toxicity. The mean Hb level was 13.9 gr/dL in vaccinated mice and 14.2 gr/dL in controls ( $P>0.05$ ), the median WBC count was 5135/ll in vaccinated mice and 6357//ll in controls ( $P>0.05$ ), the median platelet count was 1128000/ll in vaccinated mice

and 1020000/ll in controls ( $P>0.05$ ). In conclusion, vaccination with WT1 protein induces a significant cytotoxic response and a potent antibody response. This results, at least in mice, in a significant reduction of the tumor burden. The median reduction of the volume of the tumor after 8 weeks of vaccination is 62%. (Fig. 1 panel B). This strategy may allow to overcome some of the limits associated with peptide vaccination including the restriction of the HLA typing of the patient and the prevalent T CD81 response. This strategy allows to exploit the whole reactive potential of the immune system, both cytotoxic and humoral.

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## Figure Legend

Figure 1. Panel A: Scheme of vaccination. Panel B: Response in terms of tumor burden in vaccinated mice (ii) in which the tumor is undetectable compared to control mice (i) which developed a measurable tumor mass of 1.5 cm after 8 weeks from the first vaccination. Panel C: Dot blot analysis for the detection of specific antibodies against WT1. The analysis has been performed after 8 weeks from the first vaccination. Panel D: Quantification of the dot blot results. Panel E: <sup>51</sup>Cr release test for the evaluation of cytotoxicity. Panel F: Evaluation of organ toxicity before and after vaccination, respectively, in lymphonode (a,b), spleen (c,d), kidney (e, f), and ovary (g,h).



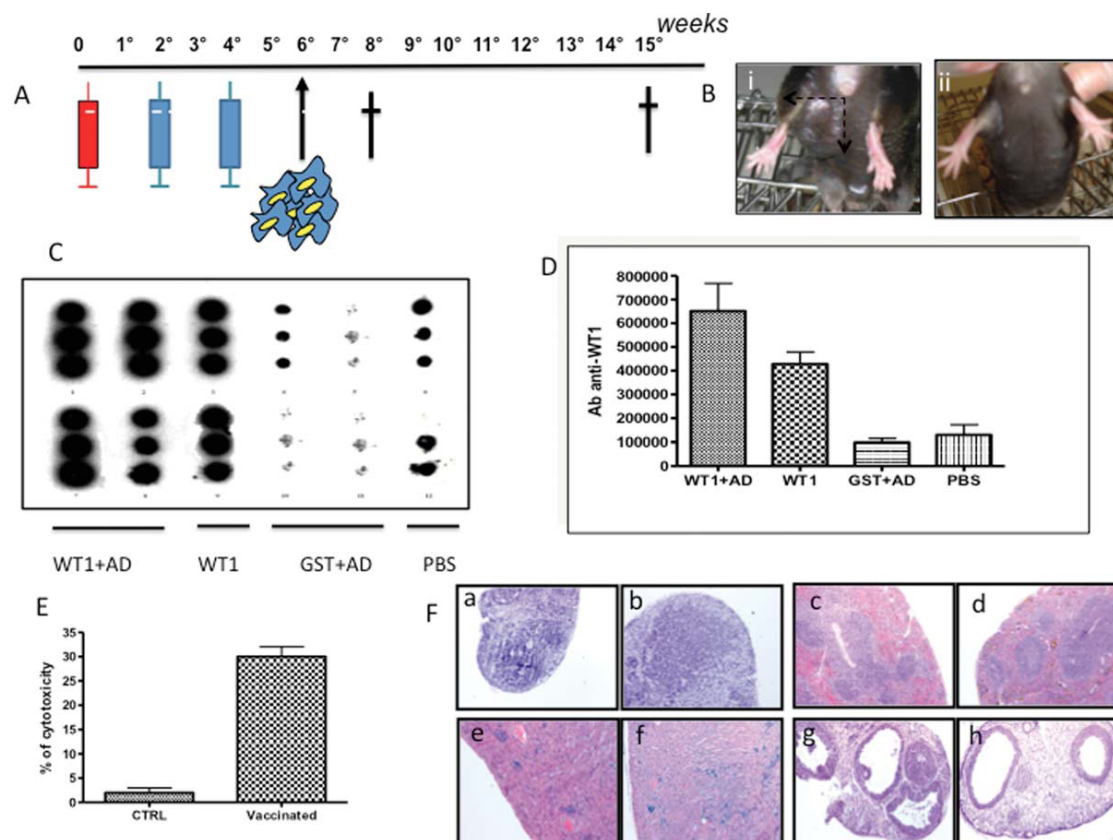


Figure 1.